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Prevalence of genetic susceptibility for breast and ovarian cancer in a non-cancer related study population: secondary germline findings from a Swiss single centre cohort

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Abstract: **BACKGROUND** Since the advent of high-throughput sequencing technologies, organised germline screening, independent of the personal and family cancer history, has been frequently proposed. Since ethnic and geographic populations significantly differ in their mutation spectra and prevalence, one critical prerequisite would be the knowledge of the expected carrier frequencies. **OBJECTIVE** For the first time, in a retrospective non-cancer related cohort from a single Swiss genetic centre, we systematically assessed the prevalence of secondary findings in 19 genes (BRCA1/2 plus 17 non-BRCA genes) previously designated by the US National Comprehensive Cancer Network (NCCN) for hereditary breast and ovarian cancer (HBOC) germline testing. **DESIGN** A total of 400 individuals without a cancer diagnosis undergoing whole-exome sequencing (WES) analysis for neurodevelopmental disorders (NDDs) from 2015 to 2017 at IMG Zurich were included after quality assessment. Among these, 180 were unaffected parental couples, 27 unaffected parental singles and 13 NDD index patients (mean age 43 years). The majority of the cohort was of Caucasian ethnicity (n = 336, 84.0%) and of Northwest European ancestry (n = 202, 50.5%), for 70 of whom (42.5%) an autochthonous Swiss descent was assumed. For WES filtering of rare, potentially actionable secondary variants in HBOC genes, an overall minor allele frequency (MAF) below 0.65% was used as cut-off. Each rare variant was manually evaluated according to the recommended ACGM-AMP standards, with some adaptations including ‘‘hypomorphic’’; as an additional distinct pathogenicity class. **RESULTS** Overall, 526 rare secondary variants (339 different variants) were encountered, with the BRCA1/2 genes accounting for 27.2% of the total variant yield. If stratified for variant pathogenicity, for BRCA1/2, three pathogenic variants were found in three females of Italian ancestry (carrier frequency of 0.8%). In the non-BRCA genes, five carriers of (likely) pathogenic variants (1.3%) were identified, with two Swiss individuals harbouring the same CHEK2 Arg160Gly variant known to be recurrent among Caucasians. Hence, the overall carrier rate added up to 2.0%. Additionally, seven various hypomorphic HBOC predisposing alleles were detected in 22 individuals (5.5%). **CONCLUSION** We provide the first evidence of a high prevalence of HBOC-related cancer susceptibility in the heterogeneous Swiss general population and relevant subpopulations, particularly in individuals of Italian descent. These pioneering data may substantiate population-based HBOC screening in Switzerland.

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Prevalence of genetic susceptibility for breast and ovarian cancer in a non-cancer related study population: secondary germline findings from a Swiss single centre cohort

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Summary

BACKGROUND: Since the advent of high-throughput sequencing technologies, organised germline screening, independent of the personal and family cancer history, has been frequently proposed. Since ethnic and geographic populations significantly differ in their mutation spectra and prevalence, one critical prerequisite would be the knowledge of the expected carrier frequencies.

OBJECTIVE: For the first time, in a retrospective non-cancer related cohort from a single Swiss genetic centre, we systematically assessed the prevalence of secondary findings in 19 genes (*BRCA1/2* plus 17 non-*BRCA* genes) previously designated by the US National Comprehensive Cancer Network (NCCN) for hereditary breast and ovarian cancer (HBOC) germline testing.

DESIGN: A total of 400 individuals without a cancer diagnosis undergoing whole-exome sequencing (WES) analysis for neurodevelopmental disorders (NDDs) from 2015 to 2017 at IMG Zurich were included after quality assessment. Among these, 180 were unaffected parental couples, 27 unaffected parental singles and 13 NDD index patients (mean age 43 years). The majority of the cohort was of Caucasian ethnicity ($n = 336$, 84.0%) and of North-west European ancestry ($n = 202$, 50.5%), for 70 of whom (42.5%) an autochthonous Swiss descent was assumed. For WES filtering of rare, potentially actionable secondary variants in HBOC genes, an overall minor allele frequency (MAF) below 0.65% was used as cut-off. Each rare variant was manually evaluated according to the recommended ACGM-AMP standards, with some adaptations including "hypomorphic" as an additional distinct pathogenicity class.

RESULTS: Overall, 526 rare secondary variants (339 different variants) were encountered, with the *BRCA1/2* genes accounting for 27.2% of the total variant yield. If stratified for variant pathogenicity, for *BRCA1/2*, three

pathogenic variants were found in three females of Italian ancestry (carrier frequency of 0.8%). In the non-*BRCA* genes, five carriers of (likely) pathogenic variants (1.3%) were identified, with two Swiss individuals harbouring the same *CHEK2* Arg160Gly variant known to be recurrent among Caucasians. Hence, the overall carrier rate added up to 2.0%. Additionally, seven various hypomorphic HBOC predisposing alleles were detected in 22 individuals (5.5%).

CONCLUSION: We provide the first evidence of a high prevalence of HBOC-related cancer susceptibility in the heterogeneous Swiss general population and relevant subpopulations, particularly in individuals of Italian descent. These pioneering data may substantiate population-based HBOC screening in Switzerland.

Keywords: hereditary breast and ovarian cancer (HBOC), Swiss, Italian, universal screening, population-based screening, secondary findings, genetic incidentaloma

Introduction

According to the Swiss Cancer Report 2015 [1], an average of more than 6200 females are diagnosed with breast or ovarian cancer in Switzerland per year, with breast cancer being the leading cancer in women and accounting for a third of all new cancer cases. It is assumed that approximately 5–10% of breast cancers and 10–15% of epithelial ovarian cancers arise in women with an underlying monogenic hereditary predisposition, frequently caused by deleterious germline variants in the cancer susceptibility genes *BRCA1* and *BRCA2* (designated as hereditary breast and ovarian cancer [HBOC] syndrome) [2]. Assuming an average prevalence of 5%, ~2000–4000 HBOC index cases are estimated to be currently living in Switzerland, according to the CASCADE study proposal for cascade cancer predisposition screening in Switzerland [3].

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However, about one in five Swiss breast cancer patients are diagnosed when they are younger than 50 years [4, 5], which may indicate genetic susceptibility and, hence, may reflect a significant underdiagnosis of underlying germline alterations. Likewise, only an estimated 20–25% of familial breast cancer cases are explained by *BRCA1/2* pathogenic variants and >60% of the hereditary predisposition remains unexplained [6, 7]. Nevertheless, in current practice, genetic testing is usually limited to the evaluation of patients meeting strict criteria for an individual or family cancer history, as specified by various (inter-)national guidelines [8–11]. These restrictions of the testing approach, which are validated for the minimum mutation detection rate of $\geq 10\%$ as a consensus [11], have been more and more questioned by recent studies using gene-panel based diagnostic strategies [12–14]. To this end, were the US National Comprehensive Cancer Network (NCCN) HBOC susceptibility screening guideline endorsed 17 non-*BRCA* susceptibility genes, which were found to confer a high to moderate breast and/or ovarian cancer risk, partially within the context of clinically broader hereditary cancer syndromes (HCSs), such as Li-Fraumeni, Lynch and PTEN hamartoma tumour syndromes [8].

Given that HBOC patients are at high risk of cancers at a younger age and of high grade, routine screening programmes lack sufficient sensitivity for the detection of HBOC-associated lesions at early stages. Magnetic resonance imaging screening from an early age and risk-reducing measures such as chemo-prevention and prophylactic surgery, however, have been shown to lead to a substantial decrease of HBOC-associated morbidity and mortality [15, 16]. These preventive strategies, as well as options for tailored therapies such as poly-ADP-ribose polymerase inhibitors (PARPi) pharmacotherapy for *BRCA1/2*-associated malignancies [17], mean that diagnostic assessment for HCSs plays a crucial role in oncological care.

Therefore, since the advent of the high-throughput sequencing technologies, organised population-based screening for *BRCA1/2* for populations harbouring a high pathogenic variant burden has been proposed several times. However, knowledge of the expected carrier frequency and possible recurrent (founder) mutations would be a critical prerequisite to giving precise estimations about the predictive values and determining if they are at diagnostically and economically reasonable levels [18–20].

Systematic investigations about specific carrier frequencies in the general – particularly autochthonous – Swiss population are nevertheless lacking. This paucity of reports even extends to the mutation frequency and spectrum in the setting of HBOC-affected cohorts originating from Switzerland and its different language areas, although these have been well studied in other, also non-Caucasian, populations.

This scarcity of studies prompted us to retrospectively assess secondary findings in the 19 NCCN-designated HBOC-related genes in a Swiss monocentric cohort of 400 individuals, almost half of whom were of assumed autochthonous Swiss descent, previously undergoing non-cancer related whole-exome sequencing (WES) analysis. Since these findings were not related to the initial reason for referral, but were identified via proactive searching, the term “secondary findings” was used throughout this study

[21, 22]. Hence, for the first time, we provide data on the prevalence of HBOC-related genetic alterations in the general population in Switzerland, which may provide a first data basis for specifying carrier rates for genetic counselling or future cancer screening programmes. The present study indicated an unexpectedly high carrier frequency for genetic variants predisposing to HBOC, especially among the Swiss subpopulation of Italian descent.

Material and methods

Study population

WES data with consent for secondary analyses from 404 subjects enrolled between 2015 and 2017 in our local monocentric study on genetic causes of neurodevelopmental disorders (NDDs) were available for this study. As described below, the WES datasets of four subjects were excluded because of insufficient data quality. The study population therefore consisted of 400 independent individuals (198 males and 202 females; median age 43 years), of whom 180 (90.0%) were parental couples not affected by NDDs, 27 (6.8%) parental singles, and 13 (3.3%) NDD index patients (table 1). For five of these index patients, genetic NDD diagnosis had been established; in eight index cases, the definitive aetiology remained unclear. For the latter, hitherto known NDD-associated tumour syndromes including Fanconi anaemia could be virtually excluded by initial phenotype-driven and panel-agnostic WES, and chromosomal microarray analyses (CMA) (Affymetrix CytoScan[®] HD or 2.7M arrays) [23, 24]. In none of the investigated individuals was a personal cancer history documented; in 28 individuals (7.0%), however, single or multiple relatives of different relationship degrees were reported to be affected by various cancer entities (supplementary table S1, appendix 1). The study cohort mostly included Caucasians ($n = 336$, 84.0%) of North West European ancestry ($n = 202$, 50.5%), among whom 170 (42.5%) were assumed to be of autochthonous Swiss descent. Non-Caucasians ($n = 64$, 16.0%) predominantly originated from the Middle East / North Africa ($n = 35$; 8.8%). Consanguinity was noted for 10.0% of individuals with a degree of relationship ranging from the sixth to the third degree. Genetic testing was part of a research project approved by the local ethics committee. Written informed consent for all kinds of scientific research questions, as well as for publication of clinical information, was obtained from participants.

Whole-exome sequencing data analysis

WES analyses was performed on DNA extracted from peripheral blood lymphocytes using Agilent (Santa Clara, CA, USA) SureSelect XT Clinical Research Kit (v5 or v6) for capturing, followed by bi-directional paired-end sequencing of 125 base pairs (bps) on a HiSeq2500 platform (Illumina, San Diego, California, USA) and alignment to the hg19 reference genome using NextGENe software (Softgenetics, State College, Pennsylvania, USA). Cut-off values for alternate read fraction and minimum coverage were 16% of supporting reads and 20 \times read depth, respectively. WES data with less than 85% of the exome covered at $\geq 20\times$ read depth were considered to be of insufficient quality. Accordingly, WES datasets of four subjects were excluded from this study in the course of quality

control. In the remaining 400 WES datasets, on average 95.9% of the WES targeted bases were covered with $\geq 20\times$ reads (87.1–98.9%) yielding an average read depth of $232\times$ (72–635 \times) (supplementary figure S1, appendix 4). For the present investigation, the coding regions including 12 flanking intronic base pairs (CDS ± 12 bps) of 19 genes related to HBOC syndrome, namely *BRCA1/2* plus 17 non-*BRCA* genes specified by the NCCN guideline "Genetic/Familial High-Risk Assessment: Breast and Ovarian" [8] (v.2.2017; see fig. 1B), were evaluated using the NextGENe software. In our exploratory preliminary analysis of variants with a minor allele frequency (MAF) $\leq 2\%$, the low-risk *BRCA2* nonsense allele NM_000059.3:c.9976A>T was the sequence alteration showing the highest overall MAF of 0.65% and that still had evidence of a potential clinical effect. An overall MAF threshold of $\leq 0.65\%$ (gnomAD database, v2.0 and later) was thus set to define rare variants potentially predisposing to HBOC. In order to ascertain sequence quality and confidence, each variant was visually validated and those considered true positives all had a NextGENe overall mutation score above the default quality threshold (≥ 12). The annotation of the filtered sequence variants was performed using NextGENe and Alamut Visual software (v.2.10; Interactive Biosoftware, Rouen, France) or by manual curation (table S2, appendices 2A and 2B).

For variant annotation and pathogenicity assessment, the web resources as detailed at the end of the article were used.

Variant classification and Sanger sequencing validation

The clinical relevance of the identified sequence variants was manually classified according to the current standards

recommended by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG-AMP) [25], with some adaptations. The variants were evaluated using a five-tier scheme and categorised as "pathogenic" (P), "likely pathogenic" (LP), "hypomorphic" (HM), "variant of uncertain significance" (VUS) and "(likely) benign" (B/LB) with the ACMG-AMP pathogenicity categories "benign" (B) and "likely benign" (LB) being subsumed to "(likely) benign" for simplicity, and a novel category "hypomorphic" being introduced. Given the empirical pathogenic variant burden in the Exome Aggregation Consortium (ExAC) database [26], the frequency levels "within pathogenic range" (≤ 8 total alleles in the ExAC database; corresponding to the moderate ACMG-AMP "PM2" criterion), "somewhat high" (>8 total alleles), "high" (overall MAF $>0.1\%$), and "very high" (overall MAF $>0.5\%$) were introduced as proposed by Nykamp et al. [27] for genes with autosomal-dominant inheritance. Also according to Nykamp et al., the MAF levels "high" and "very high" (overall MAF $>0.1\%$) were considered sufficient criteria for B/LB classification if there existed no additional corroborating evidence for variant pathogenicity. Variants were classified as hypomorphic if they had been recurrently observed and shown a low to moderate association with cancer in large case-control studies, with evidence for functional damaging effects but controversial assessment in the literature. Of note, the classification criteria proposed by the ENIGMA consortium for *BRCA1/2* variants are solely intended to differentiate germline high-risk *BRCA1/2* variants from those with low or no risk. These guidelines are therefore explicitly not intended to evaluate and classify non-high-risk variants associated with an intermediate or moderate risk (ENIGMA *BRCA1/2* Gene Variant Classification Criteria, v2.5.1., 29 June 2017). In the absence of contradictory evidence, vari-

Table 1: Detailed demographic and recruitment data for all enrolled subjects.

Total no. of investigated individuals		400			
Median age		43 years			
		n	%		
Sex	Male	198	49.5		
	Female	202	50.5		
Analysed by WES	Parental couples	180/220 (360/400)	81.8 (90.0)		
	Parental singles	27/220 (27/400)	12.3 (6.8)		
	NDD index singles*	13/220 (13/400)	5.9 (3.3)		
Consanguinity of parental couples		20/180 (40/400)	11.1 (10.0)		
Ancestry [†]	Caucasian	All	336	84.0	
		North West European	All	202	50.5
			Autochthonous Swiss [‡]	170	42.5
		South European	117	29.3	
		East European	17	4.3	
	Non-Caucasian	All	64	16.0	
		Middle Eastern / North African	35	8.8	
		East Asian	1	0.3	
		South Asian	20	5.0	
		Sub-Saharan African	8	2.0	
American	7	1.8			

NDD = neurodevelopmental delay; WES = whole-exome sequencing * No evidence of a NDD-associated tumour disposition syndrome. Diagnosis had been established for five index patients (MIM-number of the respective phenotype and the aetiological gene in brackets): ID 65891 (MIM# 300055, *MECP2*); ID 71642 (MIM# 607313, *ROBO3*); ID 73869 (MIM# 604804, *CDK5RAP2*); ID 74275 (MIM# 613382, *PTHLH*); ID 80540 (MIM# 615656, chromosome 15q11.2 microdeletion syndrome). For eight index patients, the definitive genetic diagnosis remained unclear (cardinal phenotypic features in brackets): ID 57429 (NDD, myelination defects, hypotonia, hypoplasia of abdominal wall muscles, pectus excavatum, ataxia/athetosis, dysmorphism); ID 62805 (borderline macrocephaly/macrosomia, speech delay, attention-deficit disorder); ID 66856 (septo-optic dysplasia); ID 68241 (NDD, macrosomy, behavioural problems); ID 68355 (NDD); ID 72678 (NDD, macrocephaly, dissociated cognitive dysfunction, language-acquisition disorder); ID 75672 (recurrent generalized fever-associated seizures); ID 75676 (language-acquisition disorder, macrocephaly, behavioral problems, learning disorder) † In the case of mixed ancestry, multiple ancestry labels per individual ‡ Self-reported Swiss descent, with at least one parental side originating from Switzerland

ant classifications reviewed by international expert consortia [28–32] were used as a stand-alone criterion. Variant interpretations provided in locus-specific databases (LSDBs) were taken into account for pathogenicity assessment only if they were reviewed by multiple submitters without interpretation conflicts (“two-star” review status in the NCBI ClinVar database; corresponding to the supporting ACMG-AMP “BP6” and “PP5” criteria). For the evaluation of computational evidence data, a prediction consensus was built considering seven functional and conservation prediction algorithms (see web resources). To this end, CADD Phred and GERP_RS scores of ≥ 20 and > 2 were set as cut-off values for deleteriousness, as suggested elsewhere [33]. The prediction consensus was assigned as “damaging” (corresponding to the supporting ACMG-AMP “PP3” criterion) if at least six out of seven prediction algorithms indicated a deleterious variant effect. Variants for which 1/7 or none of the predictions were damaging were assumed to be neutral and considered to meet the supporting ACMG-AMP criterion “BP4”; any other cases were categorised as “undetermined” (for further details, see legend to table S2 in the appendix). Likewise, potential splice alterations at the proximal canonical splice site and at *in silico* predicted cryptic splice donor/acceptor sites (CSDS/CSAS) were monitored for all filtered variants by using a prediction consensus of four different splicing algorithms (see web resources). A variant was predicted to induce a potential splicing defect (corresponding to the supporting ACMG-AMP “PP3” criterion) if at least three algorithms scored above the relative cut-off values established for the

score differences between wild-type and variant. For these difference scores, algorithm-specific thresholds were used as proposed by Tang et al. [34] and Baert et al. [35] and detailed in table S3 (appendix 3).

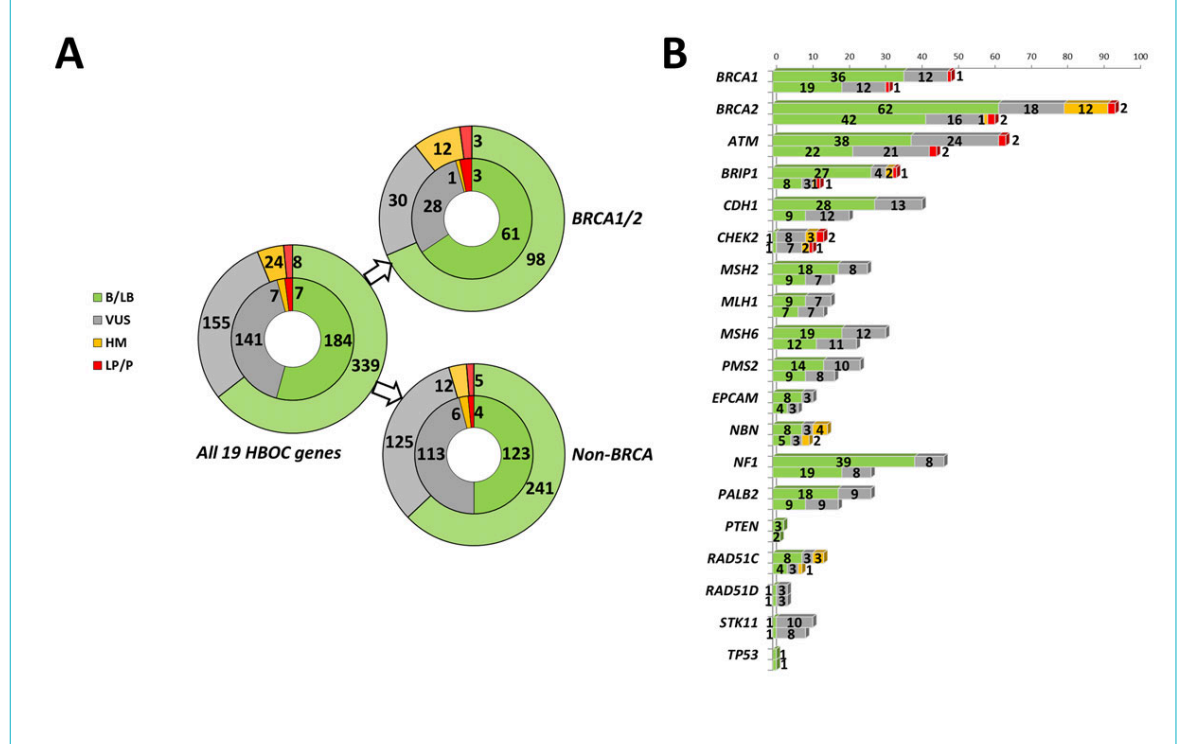
All variants finally classified as (likely) pathogenic were confirmed by bi-directional Sanger sequencing on an ABI3730 capillary sequencer (Applied Biosystems) using the SequencePilot v.4.4.0 software (JSI medical systems, Ettenheim, Germany) for evaluation.

Results

Overall, WES data evaluation for the 19 HBOC-related genes investigated identified 339 different rare variants (MAF $\leq 0.65\%$) with an absolute yield of 526 rare sequence alterations. With regard to the latter, the *BRCA1* and *BRCA2* genes accounted for 9.3% and 17.9% (combined fraction of 27.2%) of all detected rare variants, respectively (fig. 1). Among a total of 383 rare non-*BRCA* variants, including 246 different variants, most sequence alterations affected the *ATM* (12.2%) and *NF1* genes (8.9%), followed by the *CDH1* gene (7.8%). The majority of variants were missense (59.5%) followed by synonymous (26.8%), non-coding (9.9%), nonsense (2.5%), and frameshift sequence variations (1.0%) (table S2, appendix 2).

According to our variant classification, a total of eight secondary LP/P findings were found (1.5% of all detected variants), among which three (0.6%) affected *BRCA1/2* and five (1.0%) the non-*BRCA* genes, with the *CHEK2* Arg117Gly variant observed in two unrelated individuals.

Figure 1: Distribution and classification of rare variants in HBOC-related genes. **(A)** Distribution of the secondary variants detected in the 19 NCCN-designated genes related to HBOC with stratification into the *BRCA1/2* and non-*BRCA* genes and the different pathogenicity classes. In the outer circles, the absolute variant number is given; in the inner circles, the number of the different variants is shown. **(B)** Detailed pathogenicity class distribution for each evaluated gene with the absolute variant counts (upper bars) and the number of different variants (lower bars). For clarity purposes, no difference was made for the pathogenicity classes benign/likely benign and likely pathogenic/pathogenic. B = benign; HBOC = hereditary breast and ovarian cancer; HM = hypomorphic; LB = likely benign; LP = likely pathogenic; P = pathogenic; VUS = variant of unclear significance



Additionally, we detected seven different hypomorphic variants with the recurrent low-risk *BRCA2* c.9976A>T nonsense allele accounting for one half of all hypomorphic findings (12/24 HM variant carriers). Furthermore, about one third of all rare variants were VUSs (155/526, 29.5%), of which 80.6% (125/155) were detected in the non-*BRCA* genes, particularly in *ATM*, *CDH1*, and *MSH6*. The highest fraction of VUSs relative to the overall gene-specific variant yield was found for *STK11*. In contrast, both the *TP53* and *PTEN* genes showed very few sequence variations, which were all classified as (likely) benign. Among the total of 155 VUSs, 14.2% (22/155) were novel (previously unreported in LSDBs and reference populations), whereas 8.4% (13/155) were viewed as a VUS by international expert panels; an additional 36.8% (57/155) were consistently classified as VUSs by multiple submitters to the NCBI ClinVar database (“two-star” review status). Moreover, 117 (34.5%) different variants were known to the Human Gene Mutation Database (HGMD), among which 19 (5.6%) and 90 (26.5%) variants were tagged as “DM” (disease-causing mutations) and “DM?” (questionable disease-causing mutations), respectively.

Among the eight LP/P findings, half of the variants were likely gene-disrupting and half were missense (table 1). Apart from a novel *ATM* Glu2156* nonsense variant found in a 39-year-old Czech female, all variants had been previously described in the literature and/or LSDBs. For the *BRCA1/2* genes, these included the recurrent high-risk *BRCA1* c.181T>G European founder allele [36, 37] and two *BRCA2* single nucleotide duplications, both of which are annotated as pathogenic in the BRCA Exchange or BRCA Share database. Interestingly, all three *BRCA1/2* pathogenic variants were identified in middle-aged females of Italian ancestry, each of whom had no evidence of personal or family history of cancer. The non-*BRCA*-genes included the recurrent Caucasian *CHEK2* c.349A>G [p.(Arg117Gly)] variant known as a moderate risk allele for breast cancer [38, 39], which was carried by two unrelated subjects originating from the German-speaking part of Switzerland. As for *BRCA1/2*, in none of the non-*BRCA* mutation carriers any personal and family history of cancer was reported. Of note, four of these LP/P variants were transmitted to the index offspring, for all of whom an unrelated genetic lesion underlying the particular NDD phenotype had been previously elucidated. The genetic consultants of the adult carriers of a LP/P variant were informed in accordance with the given consent for genetic counselling.

Regarding the HM variants, the recurrent Lys3326* nonsense variant within the last *BRCA2* exon, for which case-control and genome-wide association studies suggested a mildly increased risk for HBOC [40, 41] and various other cancer entities [42], had the highest carrier rate of 2.3% in our sample (table 2). Of note, two thirds of the carriers were of autochthonous Swiss descent, which may reflect a high local prevalence in comparison with the 0.65% overall MAF. An additional 12 individuals (2.3%) harboured six further different HM sequence alterations in the *BRIP1*, *CHEK2*, *NBN*, and *RAD51C* genes. Both of the *CHEK2* variants detected [c.320-5T>A, p.(Arg180Cys)] have been considered as low penetrant breast cancer risk alleles among white European females [39, 45, 46]. Like-

wise, the *RAD51C* Gly264Ser variant has been supposed to confer low ovarian cancer risk elevation [47, 48], which was corroborated by a partial homology-directed repair deficiency *in vivo* [49, 50]. For the detected hypomorphic *NBN* missense variants [p.(Ile171Val), p.(Arg215Trp)], minor pleiotropic cancer risks have been suggested by a meta-analysis conducted by Gao et al. [51]. Table 3 summarises all hypomorphic variants detected.

For the individual variant burden, the carrier prevalence for at least one rare variant was 28.8 vs 60.8% (*BRCA1/2* vs non-*BRCA* genes) summing up to an overall carrier rate of 71% (fig. 2). In total, 2.0% of the individuals carried a LP/P variant (0.8 vs 1.3%); if broken down by sex, in the 202 women of the present cohort, the LP/P variant carrier rate was 2.5% (1.5 vs 1.0%). In 5.5% at least one HM variant was observed. The carrier frequency of at least one VUS was 32.0% (7.0 vs 27.3%), of which the vast majority (overall frequency 30.3%) harboured no additional HM or LP/P variants. Notably, this frequency included two VUS which were shared by consanguineous parental couples. In a further third of individuals (33.5%), single or multiple LB/B variants were found as the only rare sequence alteration.

Most strikingly, when considering the LP/P and HM variant burden as a whole, 7.3% of the individuals were identified to be carrier of HBOC-related germline alteration. Of note, these carrier individuals were not related to each other. Since none of the 13 NDD index patients exhibited any LP/P variant, no relevant difference in the carrier percentages existed when the entire study population was compared to the non-index proband group. Likewise, the carrier frequency did not significantly change if the analysis was restricted to the individuals of Swiss descent.

Discussion

Approaches to comprehensive population-wide genetic screening are being increasingly discussed since the advent of early high-throughput sequencing technologies. However, routine *BRCA1/2* assessment for the entire or female population is currently not recommended [8, 9, 61, 62]. Nevertheless, according to the HBOC guideline by the Swiss Group for Clinical Cancer Research (SAKK), individuals of Ashkenazi Jewish heritage should be referred for genetic testing, irrespective of their personal or family cancer history [9]. For the expansion to further ethnicities, as recently proposed by Lasker awardee Mary-Claire King [61], knowledge about expected carrier frequencies and possible mutation recurrences among the population of interest is critically required [19].

In contrast to the traditional estimates of *BRCA1/2* mutation carrier prevalence, which range from 0.2% to 0.3% (1:300 to 1:500) in the general female population [62], we found in our total cohort a significantly higher carrier rate of 0.8%. However, recent empirical data from the ExAC database indicate a *BRCA1/2* mutation population frequency of 0.41% in total and of 0.51% in the European population (exclusion of data from The Cancer Genome Atlas [TCGA], considering pathogenic missense mutations known to the ENIGMA database and all protein-truncating variants [PTVs] of the respective canonical transcript outside the regions distal to the last *bona-fide* pathogenic PTV and sequencing error-prone repeats) [63]. This find-

ing has very recently been confirmed by the Geisinger MyCode initiative for precision medicine, who reported in >50,000 mostly Caucasians a prevalence of 1:180 (0.56%) for Sanger-confirmed *BRCA1/2* LP/P variants when controlled for relatedness up to the third degree [64, 65].

Surprisingly, all the three *BRCA1/2* carriers in our cohort were women of Italian descent (South Italy and Sardinia). When restricted to this subpopulation the carrier rate would be as extraordinary high as 7.1% (3/42 individuals of Italian descent), which significantly differs from that of the non-Italian ancestry subgroup (0/353 with available

Table 2: Summary of all detected (likely) pathogenic variants.

Gene	HGVS cDNA change*	Predicted AA change	rs number dbSNP 147	Function	Founder mutation?	Evidence (ACMG evidence category, if meeting) [25]	Final classification	Carrier ID	Age, y	Sex	Ancestry	Variant transmitted to index?	NDD diagnosis established for affected offspring†
<i>BRCA1</i>	c.181T>G	p.(Cys61Gly)	rs28897672	Missense	Europe [36]	Classified as pathogenic by the ENIGMA consortium; one of the most recurrent founder <i>BRCA1</i> pathogenic variants in multiple European populations [1]	Pathogenic	67202	36	f	South Italian	N	N (NDD, primary microcephaly, speech delay, short stature)
<i>BRCA2</i>	c.4284 dup	p.(Gln1429Serfs*9)	rs80359439	Frameshift	Greece / South-East Europe (?) [43, 44]	Classified as pathogenic by the ENIGMA consortium	Pathogenic	47478	44	f	South Italian (Sicily)	N	N (NDD, ataxia, epilepsy)
<i>BRCA2</i>	c.4719dup	p.(Lys1574*)	None	Nonsense	n.k. to ENIGMA	Predicted LoF-variant (PVS1); unreported (PM2); known to the UMD Database with one submission as causal (PP5)	Pathogenic	75475	39	f	Italian (Sardinia)	Y	Y (MIM #300243, <i>SLC9A6</i>)
<i>ATM</i>	c.6466G>T	p.(Glu2156*)	None	Nonsense	Novel	Predicted LoF-Variant (PVS1); unreported (PM2)	Likely pathogenic	67514	39	f	Czech	N	N (NDD, microcephaly, short stature, hypotonia)
<i>ATM</i>	c.6385T>G	p.(Tyr2129Asp)	None	Missense	Founder effect n.k.	Mutational hot spot (PIK-related kinase/FAT domain) (PM1); absent in population databases (PM2); detected <i>in trans</i> with a second pathogenic variant in an A-T patient (ClinVar submission SCV000537715.1, HUG) (PM3); highly deleterious <i>in silico</i> predictions (PP3)	Likely pathogenic	62542	40	m	Turkish (Kurd)	N	Y (MIM #610536, <i>EF-TUD2</i>)
<i>BRIP1</i>	c.2684_2687del	p.(Ser895*)	rs760551339	Frameshift	Founder effect n.k.	Predicted LoF-variant (PVS1); MAF in population databases within pathogenic range (PM2); known to HGMD and ClinVar databases as (likely) pathogenic (PP5).	Likely pathogenic	68160	55	m	Swiss, German-speaking part	Y	Y (<i>MTATP6</i>)
<i>CHEK2</i>	c.349A>G	p.(Arg117Gly)	rs28909982	Missense	Recurrent among Caucasians, but founder effect n.k. [38]	Established recurrent pathogenic variant with statistically confirmed association with BC [39]	Pathogenic	73281	41	f	Swiss, German-speaking part	Y	Y (MIM #121050, <i>FBN2</i>)
<i>CHEK2</i>	c.349A>G	p.(Arg117Gly)	rs28909982	Missense				73184	59	m	Swiss, German-speaking part	N	N (severe NDD, spastic tetraplegia, epileptic encephalopathy)

? = supposed; AA = amino acid; ACMG = American College of Medical Genetics and Genomics; A-T = ataxia telangiectasia; BC = breast cancer; ENIGMA = Evidence-based Network for the Interpretation of Germline Mutant Alleles; f = female; FAT = FRAP-ATM-TRRAP; HBOC = Hereditary Breast and Ovarian Cancer; HGMD = Human Gene Mutation Database; HGVS = Human Genome Variation Society; HUG = Hôpitaux Universitaires de Genève; LoF = loss of function; m = male; MAF = minor allele frequency; N = no; NDD = neurodevelopmental delay; n.k. = not known; PIK = phosphatidylinositol kinase; UMD = Universal Mutation Database; Y = yes * Reference transcripts: *BRCA1*, NM_007294.3; *BRCA2*, NM_000059.3; *ATM*, NM_000051.3; *BRIP1*, NM_032043.2; *CHEK2*, NM_007194.3 † If NDD diagnosis is established, the phenotype MIM number and the aetiological gene is given; if not, cardinal phenotypic features are summarised

ancestry information; $p = 0.0011$, Fisher's exact test). Of note, neither the *BRCA1/2* carriers, nor the carriers of LP/P variants in non-*BRCA* genes had a documented personal or family history of cancer and therefore they are not listed in table S1.

This unprecedentedly high pathogenic variant rate in *BRCA1/2* might be explained by the small sample size, but could reflect an actually higher *BRCA1/2* mutation prevalence among subjects originating from Italy. In accordance with the latter assumption, in larger cohorts of Italian patients with familial breast/ovarian cancer, consistently high *BRCA1/2* carrier rates of up to 33.6% have been reported, with multiple, partially regionally recurring, founder mutations accounting for more than 10% of all *BRCA1/2* pathogenic variants [66–69]. In particular, in a large unselected invasive ovarian cancer series from Ontario, Canada, patients of Italian origin carried the most *BRCA1/2* pathogenic variants (20/46, 43.5%), owing in part to the recurrence of three founder mutations, which caused the authors to discuss more widespread screening among Italians [70, 71]. In line with this, the significantly higher breast/ovarian cancer incidence in Western Switzerland and Canton of Ticino (Italian-speaking) compared with the German-speaking part of Switzerland [1] gives reason to speculate about a higher *BRCA1/2* mutation burden among Italian-descent individuals. Accordingly, two of the three *BRCA1/2* pathogenic variants observed in our patients are recurrently seen in Italian breast/ovarian cancer patients. First, we found in a healthy woman from Southern Italy the *BR-*

CA1 c.181T>G [p.(Cys61Gly)] variant, which represents the second most common pathogenic *BRCA1* allele among Italian *BRCA1* families (prevalence of 3.9%) [37]. Nevertheless, this variant ubiquitously recurs in Central Europe and had been identified on a common Polish and Ashkenazi Jewish haplotype, suggesting it is an Eastern European founder mutation [72]. The second recurrent Italian variant (*BRCA2*: c.4284dup) was previously reported in a North-Italian patient with synchronous breast and ovarian cancer with a frameshift *BRCA1* mutation in double heterozygosity [73] as well as in two other Italian ovarian cancer patients [71, 74].

With regard to the non-*BRCA* genes, the comparability of our carrier rate of 1.3% is limited, because, to our knowledge, only screenings including subpanels of the 19 NCCN HBOC genes have been published so far. Most published WES screenings were for the detection of secondary findings across the ACMG-designated minimum list of 56, or nowadays 59, medically actionable genes (ACMG-56/59) [75, 76], among which nine genes only are shared with the NCCN gene panel, with important genes such as the *ATM*, *CHEK2*, and *PALB2* genes missing. These core genes show the highest mutation prevalence (2.5, 1.5 and 1.2%) in a very recent series of >5500 German *BRCA1/2*-negative breast cancer index patients [77] and also account for 1.0% of the LP/P variant yield in the present cohort of individuals without cancer. Nevertheless, ACMG-56/59-based studies in predominantly Caucasian cohorts found that the carrier rates in nine shared HBOC genes ranged from 0.3

Table 3: Summary of all detected hypomorphic variants.

Gene	HGVS cDNA change*	Predicted AA change	rs number dbSNP 147	Function	Selected case-control and functional studies (OR, Odds ratio [95%CI])	Carrier ID	Age, y	Sex	Ancestry
<i>BRCA2</i>	c.9976A>T	p.(Lys3326*)	rs11571833	Nonsense	2634 BC kindreds: OR = 1.53 (1.00–2.34) [40]; 41,081 BCAC BC cases: ORw = 1.28 (1.17–1.40), 14,514 OCAC invasive OC cases: ORw = 1.26 (1.10–1.43) [41]; 43,641 genotyped cancer patients: OR (small cell lung cancer) = 2.06 (1.35–3.16), OR (squamous cell carcinoma of the skin) = 1.69 (1.26–2.26) [42]; minor reduction of HDR activity in [52]	12 individuals			among these, eight Swiss individuals
<i>BRIP1</i>	c.139C>G	p.(Pro47Ala)	rs28903098	Missense	1882 glioma cases: OR = 3.83 (1.01–14.5) [53]; initially reported in an individual with early-onset BC and strong BC/OC family history [54, 55]; destabilisation and abolishment of the BRIP1 helicase activity in [55, 56]	71230	44	F	Dutch?
<i>BRIP1</i>	c.139C>G	p.(Pro47Ala)	rs28903098	Missense		62926	51	F	Swiss
<i>CHEK2</i>	c.320-5T>A	p.(?)	rs121908700	Noncoding	13,087 ECRIC BC cases: OR = 13.9 (1.89–101) [45]; leaky splicing defect implied by [46]	67184	48	M	Swiss
<i>CHEK2</i>	c.320-5T>A	p.(?)	rs121908700	Noncoding		75475	nk	F	Italian
<i>CHEK2</i>	c.538C>T	p.(Arg180Cys)	rs77130927	Missense	34,488 unselected white European BC patients: OR = 1.34 (1.06–1.70) [39]; intermediate DNA damage response in [57]	68159	53	F	Dutch
<i>NBN</i>	c.511A>G	p.(Ile171Val)	rs61754966	Missense	Meta-analysis of 39,731 cancer cases: OR = 3.93 (1.68–9.20) [51]; impaired DSB repair activity and increased chromosomal instability in [58]	77074	44	F	Swiss
<i>NBN</i>	c.511A>G	p.(Ile171Val)	rs61754966	Missense		77745	32	F	Serbian
<i>NBN</i>	c.511A>G	p.(Ile171Val)	rs61754966	Missense		57429	10	M	Croatian
<i>NBN</i>	c.643C>T	p.(Arg215Trp)	rs34767364	Missense	Meta-analysis of 39,731 cancer cases: OR = 1.77 (1.07–2.91) [51]; impaired DSB repair activity in [59, 60]	66310	38	M	Portuguese
<i>RAD51C</i>	c.790G>A	p.(Gly264Ser)	rs147241704	Missense	620 BC cases, 480 BC/OC cases: OR (BC/OC) = 3.44 (1.51–7.80) [48]; intermediate HDR activity [49, 50]	70861	51	F	Swiss
<i>RAD51C</i>	c.790G>A	p.(Gly264Ser)	rs147241704	Missense		68968	36	F	Armenian/Russian
<i>RAD51C</i>	c.790G>A	p.(Gly264Ser)	rs147241704	Missense		60577	39	F	Swiss

? = supposed; AA = amino acid; BC = breast cancer; BCAC = Breast Cancer Association Consortium (<http://bcac.ccge.medschl.cam.ac.uk>); CI = confidence interval; DSB = double-strand DNA break; ECRIC = East Anglia Cancer Registration and Information Centre; F = female; HDR = homology-directed repair; HGVS = Human Genome Variation Society; OC = ovarian cancer; OCAC = Ovarian Cancer Association Consortium; OR, odds ratio; ORw = OR using weighted logistic regression models * Reference transcripts: *BRCA2*, NM_000059.3; *BRIP1*, NM_032043.2; *CHEK2*, NM_007194.3; *NBN*, NM_002485.4; *RAD51C*, NM_058216.2

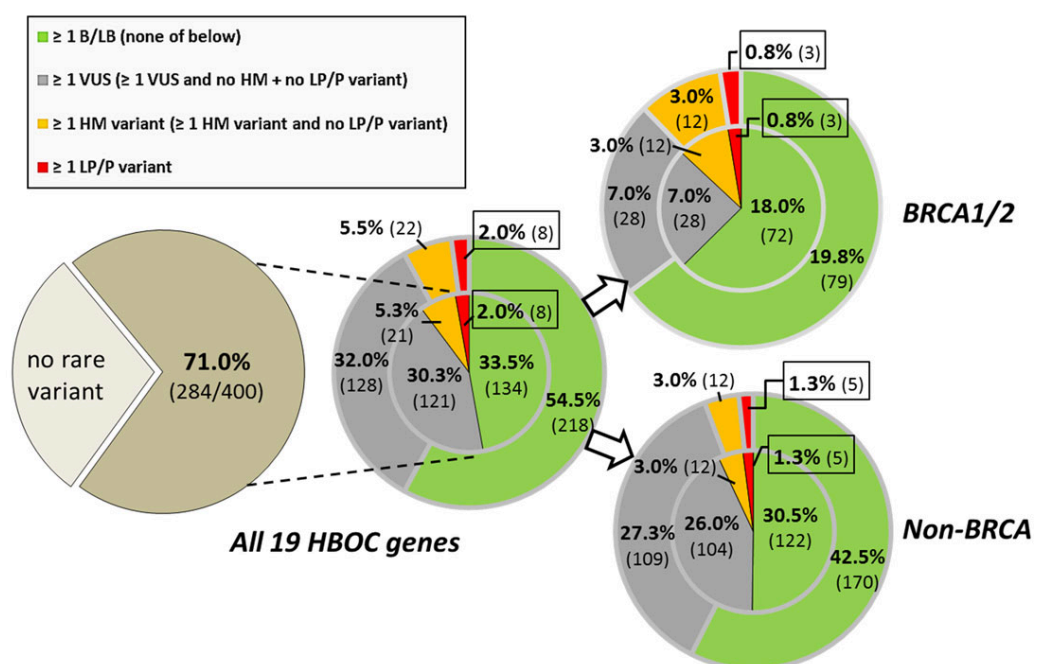
to 2.5% [78–83], with variant classification inconsistencies and other confounding factors. In a couple of pioneer papers [23, 84–86], large cohorts were screened for more expanded gene panels, including up to 16 genes [87] out of the present HBOC panel, but these studies also failed to consider, particularly, the *ATM* and *CHEK2* core genes.

Thompson et al. [88], however, screened almost 2000 cancer-free Australian women for secondary findings in a panel of 18 breast cancer-associated genes covering the non-*BRCA* core genes *ATM*, *BRIP1*, *CDH1*, *CHEK2*, *PALB2* and *TP53*, which is more suitable for the comparison with the data from our cohort. With regard to the 12 overlapping genes, the resulting prevalence was somewhat lower in their study population (1.8%) as compared with our cohort (2.0%). This also holds true when comparison is made for *BRCA1/2* (0.65 vs 0.8% in our cohort) and non-*BRCA* genes (1.1 vs 1.3%). This difference may be partly explained by the inclusion of cancer-free women exclusively aged >60 by Thompson et al., which may result in a partial depletion of highly penetrant mutations in their series (mean age in our cohort 43 years). Similarly, the prevalence of PTVs in eight non-*BRCA* core genes in control datasets derived from ExAC was 1.4% (27,173 non-Finnish European [NFE] individuals), whereas it was 0.9%, in the hypernormal FLOSSIES populations (7325 cancer-free woman of European American ancestry over 70 years of age) [77]. However, in addition to inter-study variability in sample size, demographic structure and inclusion criteria, comparability is further hampered by

considerable methodological inconsistencies in the stringency of variant filtering and pathogenicity assessment together with technical sequencing setups, and therefore data quality [31, 80–83]. As an illustration, the *BRCA1/2* mutation carrier frequency among the Finnish ExAC cohort was considerably lower than that of NFE subpopulation (0.08 vs 0.24%); inter-population differences, however, may be confounded by the contribution of Ashkenazi Jewish founder mutations present only in the NFE cohort. Likewise, there is substantial variability between the Africans, Latinos, East and South Asians with ExAC frequencies ranging from 0.16% to 0.41% among Africans to Latinos [63], which may be influenced by limitations in sample size and the fact that the existing literature is biased towards Caucasian cohorts. The latter may mean that there is insufficient evidence to classify missense variants recurring among non-Caucasians as definitely pathogenic [89].

Moreover, Maxwell et al. [63], among others, rightly pointed out that true population prevalence might be underestimated in available studies, owing to the lack of consideration of copy number variants (CNVs), which account for ~10% of all *BRCA1/2* mutations [90], and the disregard of uncharacterised but (potentially) pathogenic missense variants. Even though the present study also did not include CNVs and structural rearrangements, we assessed the clinical significance of missense variants by extensive manual curation without any database-driven pre-selection variant filtering strategy, allowing us to give a more representative reflection of the true mutation burden. Furthermore, we al-

Figure 2: Carrier frequencies in non-cancer related individuals (n = 400) for at least one rare variant (overall MAF ≤0.65) in the HBOC-genes (dark brown), stratified to the *BRCA1/2* and non-*BRCA* genes and the different pathogenicity classes (inset). Frequencies for mutually exclusive stratification categories (single count of the "highest" applicable pathogenicity category per individual) are given in the inner circle; the frequencies for non-exclusive categories (multiple categories were counted per individual if applicable) are determined separately and hence non-additive. The absolute counts are given in brackets. B = benign; HBOC = hereditary breast and ovarian cancer; HM = hypomorphic; LB = likely benign; LP = likely pathogenic; P = pathogenic; VUS = variant of unclear significance



so incorporated into our evaluation hypomorphic variants, which were found in the considerable percentage of 5.5% of the studied individuals.

In contrast to many other populations, for Switzerland as a whole, the pathogenic variant spectrum and prevalence for the HBOC-related genes even in cancer patients is far from being established. To our knowledge, hitherto only three Swiss retrospective series with up to 350 breast/ovarian cancer patients meeting stringent clinical criteria have been published, in which typical *BRCA1/2* pathogenic variant carrier rates of about 25% were detected [5, 84, 85]. Notably, these studies solely addressed *BRCA1/2* and, regardless of the heterogeneity and peculiarities of the Swiss population [86, 91], provided no specific inclusion or stratification criteria related to the ancestry of the enrolled patients. In our cohort, two unrelated individuals originating from the German-speaking part of Switzerland shared the pathogenic *CHEK2* Arg117Gly variant, which is one of the most recurrent non-founder *CHEK2* mutations among Caucasians [38]. To our knowledge, this variant has not yet been characterised in Swiss individuals. Exploratory examinations on the basis of the trio-WES data argue against a shared *CHEK2* haplotype, though only a limited number of WES covered single-nucleotide polymorphic (SNP) sites of the *CHEK2* haplotype-constituting SNP ensembles provided by Einarsdottir et al. [92] and Kaufman et al. [93] could be analysed.

In conclusion, our pioneer analyses indicate a substantial high burden of HBOC-related breast and ovarian cancer susceptibility in the heterogeneous Swiss general population and relevant subpopulations, especially in subjects of Italian descent. Whereas the LP/P variants found in 2.0% of the studied local population and 7.1% of the Italian subpopulation would warrant clinical action, the clinical implications of the hypomorphic variants detected in 5.5% of the population are currently not defined. Especially since all the LP/P variants were detected in individuals without personal or family cancer history, our data may corroborate further considerations about population-based HBOC screenings in Switzerland, particularly for individuals of Italian ancestry, and may serve as a starting point for screening in larger cohorts, preferably with genetically inferred ancestry. Organised screening may contribute decisively to the identification of the many mutation carriers who are presently undiagnosed as a result of the current restrictive testing practice and therefore excluded from referral to special HBOC management programmes at an early age. Nevertheless, the high rate of VUS detected, especially in non-*BRCA* genes, would require strict policies for non-disclosure of VUS, as well as regular and systematic reassessments.

Web resources

Population databases and datasets:

Database of Single Nucleotide Polymorphism (dbSNP), <https://www.ncbi.nlm.nih.gov/SNP>

Exome Aggregation Consortium (ExAC) Browser, <http://exac.broadinstitute.org>

Fabulous Ladies over Seventy (FLOSSIES) Database, <https://whi.color.com>

Genome Aggregation Database (gnomAD), <http://gnomad.broadinstitute.org>

NHLBI Exome Sequencing Project (ESP) Exome Variant Server, <http://evs.gs.washington.edu/EVS>

1000 Genomes (1000G) Project, <http://www.1000genomes.org>

Locus-specific databases (LSDBs):

ARUP BRCA Mutation Database, <http://www.arup.utah.edu/database>

BRCA Exchange Database, <http://brcaexchange.org>

Zhejiang University Center for Genetic and Genomic Medicine Database, <http://www.genomed.org/lovd2/home.php>

Global Variome Shared Leiden Open Variation Database (LOVD), <https://databases.lovd.nl/shared/genes>

Human Gene Mutation Database (HGMD), <https://www.hgmd.org>

International Agency for Research on Cancer (IARC) TP53 Database, <http://p53.iarc.fr>

International Society for Gastrointestinal Hereditary Tumours (InSight) Databases, <http://www.insight-database.org/genes>

Kathleen Cuninghame Foundation Consortium for Research into Familial Breast Cancer (kConFab) Database, <http://www.kconfab.org>

LOVD - Human Mismatch Repair Genes, <http://HCI-LOVD.hci.utah.edu/home.php>

Mismatch Repair Genes Variant Database, <http://www.med.mun.ca/mmrvariants>

NCBI ClinVar Database, <https://www.ncbi.nlm.nih.gov/clinvar>

NHGRI Breast Cancer Information Core (BIC) Database, <https://research.nhgri.nih.gov/bic>

Universal Mutation Databases (UMD), <http://www.umd.be>

In-silico functional prediction algorithms:

Align-Grantham Variation Grantham Deviation (GVGD), <http://agvgd.hci.utah.edu>

Combined Annotation Dependent Depletion (CADD) score, <http://cadd.gs.washington.edu/score>

EMBL-EBI InterPro (v.67.0), <https://www.ebi.ac.uk/interpro>

Genomic Evolutionary Rate Profiling (GERP++)_Rejected Substitution (RS), <http://mendel.stanford.edu/SidowLab/downloads/gerp>

MutationTaster (MutatTaster), <http://www.mutation-taster.org>

Polymorphism Phenotyping (PolyPhen)-2, <http://genetics.bwh.harvard.edu/pph2>

Sorting Intolerant from Tolerant (SIFT), <http://sift.jcvi.org>

Universal Mutation Database (UMD) predictor, <http://umd-predictor.eu/>

In-silico tools for splicing defect prediction (assessed by Alamut Visual v.2.10 software):

SpliceSiteFinder (SSF)-like, <http://www.interactive-biosoftware.com>

MaxEntScan (MES), http://genes.mit.edu/burgelab/max-ent/Xmaxentscan_scoreseq.html

Splice Site Prediction by Neural Network (NNS), http://www.fruitfly.org/seq_tools/splice.html

Human Splicing Finder (HSF), <http://www.umd.be/HSF>

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Potential competing interests

AR is director of a diagnostic laboratory offering genetic testing for hereditary cancer. The other authors declare that they have no conflict of interest.

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Appendices

Appendix 1

Table S1: Individuals with documentation of family history of cancer.

Appendix 2A and 2B

Table S2: Synopsis of all detected secondary variants including annotations and criteria for pathogenicity assessment.

Appendix 3

Table S3: Synopsis of the in-silico predictions of potentially splice-affecting variants.

Appendix 4

Figure S1: Box plot of the coverage data of all individual WES analyses.

Appendix 5

Reference list for the supplementary data appendices.

The appendices are available in separate files for downloading at <https://smw.ch/en/article/doi/smw.2019.20092/>